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The effect of feed intake containing whole cottonseed on blood parameters of Nellore bulls

[*Efeito do consumo de rações contendo caroço de algodão sobre parâmetros sanguíneos de bovinos Nelore*]

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ABSTRACT

The aim of this study was to evaluate the effect of diets containing whole cottonseed (WC) on blood biochemical parameters of bulls. Thirty bulls with 30±6 months and 382.7±28.4kg were kept in feedlot (85 days) and fed the following WC levels: 0, 2.22, 4.44, 6.66, 8.88 and 11.11%. In comparing biochemical indicators from the beginning to the end of the experiment, the control group (CG) and those fed diets containing 2.22, 8.88 and 11.11% of WC had an increase ($P<0.05$) in serum Ca concentrations (8.34±0.65−9.56±0.92mEqL^{-1}). In relation to Fe (202.79±69.04−300.04±79.88µg/dL), the CG and those treated with 6.66% WC showed an increase ($P<0.05$) in serum concentrations. As to the Mg (1.92±0.18−2.40±0.27mEqL^{-1}), groups treated with diet containing 2.22, 4.44 and 6.66% of WC had higher ($p<0.05$) concentrations at the end of the study. Regarding blood lipids, groups with diets containing 2.22; 4.44; 6.66 and 8.88% of WC increased ($p<0.05$), respectively, in concentrations of HDL, TG, VLDL and COL. The group that received 11.11% of WC increased ($P<0.05$) in the concentrations of COL and HDL. It can be concluded that diets containing WC, caused no alterations in the concentrations of blood parameters analyzed in this study, with the exception of Mg.

Keywords: bull, serum biochemical, whole cottonseed, gossypol

RESUMO

Objetivou-se avaliar o efeito de dietas contendo caroço de algodão (CA) sobre os parâmetros sanguíneos de bovinos. Trinta touros com 30±6 meses e 382,7±28,4kg foram confinados (85 dias) e receberam dietas com as seguintes proporções de CA: 0; 2,22; 4,44; 6,66; 8,88, e 11,11%. Ao se compararem as concentrações dos indicadores bioquímicos do início com as do final do experimento, foi observado que o grupo controle e aqueles com dietas contendo 2,22; 8,88 e 11,11% de CA apresentaram aumento ($P<0,05$) na concentração sérica de Ca (8,34±0,65−9,56±0,92 mEqL^{-1}). Já em relação ao Fe (202,79±69,04−300,04±79,88µg/dL), observou-se que apenas o grupo controle e aquele tratado com 6,66% apresentaram aumento ($P<0,05$) em sua concentração. Quanto ao Mg (1,92±0,18−2,40±0,27 mEqL^{-1}), os grupos tratados com 2,22; 4,44 e 6,66% de CA apresentaram maiores concentrações ($P<0,05$) ao final do estudo. Com relação aos lipídeos sanguíneos, os grupos com dietas contendo 2,22; 4,44; 6,66 e 8,88% de CA tiveram aumento ($P<0,05$), respectivamente, nas concentrações de HDL, TG, VLDL e COL. O grupo que recebeu 11,11% de CA teve aumento ($P<0,05$) nas concentrações de COL e HDL. Conclui-se que as dietas contendo CA não causaram alterações ($P>0,05$) nas concentrações dos parâmetros sanguíneos analisados, com exceção do Mg.

Palavras-chave: touro, bioquímica sérica, caroço de algodão, gossipol

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INTRODUCTION

The use of cottonseed in ruminant feeds has aroused great interest, mainly because it is an important source of energy, proteins and supplemental to fodder. There is also a positive impact on herd productivity (Gomes *et al*., 2014) observed.

The high energy content, attributed to the oil found in oilseeds, is one of the limitations to the use of cottonseed, because the excess of unsaturated fatty acids may cause changes in ruminal fermentation, due to suppression of cellulolytic bacteria and methanogenic activities (Van Soest, 1994). The use of high quantities of food rich in lipids in ruminant diets can damage the digestibility of fibers in the rumen, putting animal performance at risk (Fernandes *et al*., 2002).

However, the main problem that has limited the use of cottonseed in ruminant feeds is the presence of gossypol, which is a toxic polyphenol found naturally in the pigment glands of whole cottonseed. Besides being a reactive compound, it quickly binds to minerals and amino acids when it is in its free form (Guedes and Soto-Blanco, 2010). The factors that predispose ruminants to gossypol toxicity are: age, duration of intake, rumen function and protein and mineral contents of the feed. For signs of gossypol poisoning to happen it is necessary that its intake occurs for several weeks or months, because the effect is cumulative (Rogério *et al*., 2003).

For general assessment of animal health, Carlson (1993) recommended a clinical chemistry panel in which are included blood dosages of electrolytes. For assessment of the nutritional "status" of mineral elements, changes in electrolytes in the blood have proved to offer a more valuable and decisive diagnostic than their concentrations in the diet ingested (Morais *et al*., 2000). Laboratory tests can help diagnosing pathologies and evaluating the reproductive and nutritional capacity of cattle. The blood biochemical composition accurately reflects the metabolic state of animal tissues, and may evaluate lesions, disturbances in the functioning of organs, animal adaptation to nutritional challenges, specific physiological and metabolic imbalances (González and Scheffer, 2002).

Due the importance of serum biochemistry as a diagnostic tool, this research is outlined with the aim of evaluating the effect of adding different levels of cottonseed to the diet of feedlot-finished Nellore cattle on serum biochemical parameters during the 85-day experimental period.

MATERIAL AND METHODS

This study was conducted from August to November, in the municipality of Campo Verde, 140km away from Cuiabá, Mato Grosso State (15°32’48’’S 55°10’08’’W). The Research Ethics Committee of the University of Cuiabá – CEP/UNIC approved the study under registration No. 60 CEP/UNIC - Protocol 2010-061.

Thirty Nellore bulls with an average age of 30±6 months and body weight of 382.7 ± 28.4kg were used for the feedlot operation, which lasted 85 days. The animals were identified, vaccinated, and subjected to parasite control before the experimental period.

The bulls were randomly distributed into six feedlot stalls with an area of 100m^2 each, containing a feed bunker and drinker. The five animals in each stall received diets with increasing amounts of whole cottonseed as follows: 0; 2.22; 4.44; 6.66; 8.88; and 11.11kg/100kg of dry matter (Tab. 1). The diets were formulated according to the requirements for bulls growing at a rate of 1.2kg/animal/day. All diets had the same energy and nitrogen contents as established by Valadares Filho *et al*. (2006). The animals were fed three times a day, at 07h00min, 12h00min, and 17h00min.

The mean content of free gossypol in the study of cottonseed was determined by high-performance liquid chromatography using the methodology described by Romero *et al*. (2011). The samples had a mean free gossypol content of 4.5g/kg of cottonseed. The animals would need to consume a mean of 20.4kg/animal/day of natural matter intake to keep the mean free gossypol content in different treatments between 0 and 5g of free gossypol/animal/day.

Table 1. Proportion of the ingredients of the experimental diets based on dry matter and bromatological composition

Ingredients (%)*	Whole cottonseed (%)					
	0	2.22	4.44	6.66	8.88	11.11
Corn silage	50.00	50.00	50.00	50.00	50.00	50.00
Whole cottonseed	0.00	2.22	4.44	6.66	8.88	11.11
Ground corn	40.46	39.06	39.63	36.22	34.8	33.39
Soybean meal	8.54	7.72	6.93	6.12	5.32	4.50
Mineral mixture**	1.00	1.00	1.00	1.00	1.00	1.00
Crude protein(1)	11.00	11.00	11.00	11.00	11.00	11.00
Ether extract(1)	3.25	3.60	3.95	4.30	4.65	5.00
Neutral detergent fiber(2)	34.61	35.26	35.9	35.55	37.2	37.85
Total digestible nutrients(3)	71.20	71.10	71.10	71.07	71.03	71.00

*2.5 g of Rumensin® per animal/day and approximately 90 g NaCl per animal/day were added to the diets.
**sodium chloride = 27.02 %; dicalcium phosphate = 67.30 %; manganese sulfate = 1.28 %, zinc sulfate = 3.67 %, copper sulfate = 0.67 %, cobalt sulfate = 0.04 %; sodium selenite = 0.003 % and potassium iodate = 0.002 %.
(1)Determined by the method described by Silva and Queiroz (2002).
(2)Determined by the method described by Van Soest *et al*. (1991).
(3)Values estimated according to Capelle *et al*. (2001).

The leftovers in the troughs were weighed daily to determine intake. Diet and leftover samples from each treatment were collected daily and combined weekly to compose a representative weekly sample for future analysis. These samples were then frozen to -18°C and used for determining the dry matter content of the different treatments. The mean daily natural matter intake, whole cottonseed intake, and gossypol intake of animals in the different treatments were calculated at the end of the experiment. The average consumption of natural matter from cattle subjected to the control diet and to diets containing 2.22; 4.44; 6.66; 8.88 and 11.11 % of cottonseed were, respectively, 22.0, 22.1, 20.9, 22.4, 19.4 and 20.7 (kg/animal/day) for an average consumption of cottonseed of 0, 0.24, 0.46, 0.72, 0.85 and 1.13, (kg/animal/ day) and an average consumption of free gossypol of 0, 1.08, 2.07, 3.26, 3.81 and 5.05 (g/animal/day), respectively.

For biochemical analysis of the parameters of the cattle, blood samples were collected by puncture into the external jugular vein, at the beginning (d=0) and at the end (d=85) of the experiment, using a *vacutainer* system in siliconized glass tubes and the samples were kept at room temperature to facilitate clot retraction. The following centrifuged real power equal centrifugation at 1,000*g* for 15 minutes for the occurrence of an adequate syneresis of the clot. The serum was separated by aspiration and kept and stored at -20 until the time of analysis.

Biochemical analyzes were performed on the serum obtained after the centrifugation process, in an automated apparatus, Model BS120 Chemistry Analyzer, of the following serum components: calcium (Ca), phosphorus (P), potassium (K), iron (Fe), sodium (Na), magnesium (Mg), chlorine (Cl), cholesterol (COL), triglyceride (TG) and high density lipoproteins (HDL). The analyses were performed at the Laboratory of Experimental Nutrition of the College of Nutrition of the Fluminense Federal University by *BioClin*® (Basic - Quibasa Chemistry, BH) commercial kits. Cholesterol concentrations of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated according to methodology described by Friedewald *et al.* (1972).

The study variables were subjected to analysis using the statistics software for Windows XLSTAT version 2015.1.03. The data was presented by arithmetic mean and standard deviation. The variables, initially, were submitted to normal analysis using the Kurtosis test to see if the data would present, or not, parametric distribution. The variables with parametric distribution were subjected to analysis of variance using the Tukey test as a post-test. Paired *t* Student Test was also performed to compare the means from the beginning and the end of treatment in each serum biochemical parameters evaluated in this study. The significance level accepted in both tests was 5%.

RESULTS AND DISCUSSION

Table 2 shows the results of the biochemical parameters of cattle fed diets containing different concentrations of cottonseed. There was no difference in serum concentration of Ca, P, K, Fe, Na, Mg and Cl between the different groups, both at the beginning and end of the study (Tab. 2).

Table 2. Serum concentrations of electrolytes in cattle fed diets containing different cottonseed concentrations at the beginning (B) and end (E) of the experiment

	% Whole cottonseed in diet *					
	0	2.22	4.44	6.66	8.88	11.11
Ca(B)	7.90±0.70a	8.33±0.67a	8.37±0.74	8.60±0.8	8.35±0.83a	8.50±0.46a
Ca(E)	9.53±1.10b	9.40±0.89b	9.86±0.59	9.12±1.46	10.08±0.17b	9.46±0.95b
P(B)	5.00±0.44a	4.40±0.66a	4.27±0.32a	4.90±0.75a	4.48±0.33a	4.33±0.35a
P(E)	6.85±0.66b	6.75±1.15b	6.18±0.71b	6.86±1.33b	6.28±0.64b	6.56±0.40b
K(B)	4.77±0.61	4.70±0.53	4.57±0.40	4.97±0.76	5.20±0.12	5.00±0.36
K(E)	5.03±0.33	4.83±0.26	5.00±0.44	4.90±0.71	5.15±0.21	4.96±0.26
Fe(B)	175.00±43.51a	216.33±100.2	202.00±86.76	164.00±59.41a	227.00±29.97	224.33±114.10
Fe(E)	331.33±81.13b	283.75±53.58	297.00±64.04	329.80±80.12b	258.75±116.50	300.60±99.59
Na(B)	144.00±13.08	145.00±12.53	142.67±10.79	143.67±10.07	151.00±7.62	141.67±5.51a
Na(E)	154.25±3.50	153.75±6.40	155.60±3.44	148.60±14.24	155.00±0.82	151.00±5.68b
Mg(B)	1.93±0.29	1.97±0.12a	1.97±0.21a	1.80±0.17a	2.03±0.13	1.80±0.12
Mg(E)	2.53±0.33	2.55±0.19b	2.32±0.28b	2.32±0.22b	2.45±0.41	2.32±0.22
Cl(B)	95.33±3.06	98.67±5.13	96.00±1.00	97.00±2.65	98.00±2.16	93.67±2.08a
Cl(E)	102.25±2.06	100.25±4.72	99.40±5.32	100.50±2.08	103.00±4.08	98.60±2.70b

a,b: different letters in the same column denote significant differences ($p<0.05$) between the results from the beginning compared to the end of the experiment, within each group - Paired *t* Student Test.
* In ANOVA treatments with different cottonseed concentrations showed no significant difference ($p>0.05$).
Normal reference values by Kaneko *et al*. (2008): Ca = 9.7-12.4 $mEqL^{-1}$; P = 5.6-6.5 $mEqL^{-1}$; K = 3.9-5.8 $mEqL^{-1}$; Fe = 57-162 µg/dL; Na = 132-152 $mEqL^{-1}$; Mg 1.8-2.3 $mEqL^{-1}$; Cl = 97-111 $mEqL^{-1}$.

However, when comparing the concentrations of biochemical indicators from the beginning of the study to the ones found at the end, within each group, it was observed that the animals that received the control diet, and with 2.22, 8.88 and 11.11% of cottonseed, presented an increase ($P<0.05$) in serum calcium concentration. This suggests that the inclusion of whole cottonseed in the cattle diet has no effect on serum calcium concentration. In relation to iron, however, only the control group and those treated with 6.66 % of cottonseed showed an increase ($P<0.05$) in their serum concentration. As for magnesium, groups treated with diet containing 2.22, 4.44 and 6.66% of cottonseed in the diet had higher ($P<0.05$) concentrations at the end of the study. This suggests that diets containing up to 6.66 % of cottonseed can increase the bioavailability of magnesium, since it was observed the rise in concentrations of this electrolyte until this ratio. The diet containing higher proportions of cottonseed may reduce its bioavailability. This may occur by increasing the gossypol content in the diet, capable of binding to minerals and reduce their absorption.

By comparing the initial serum values to the ones found at the end of the study, it was observed that serum phosphorous concentration increased ($P<0.05$) in all groups, indicating that the increase in serum phosphorus concentration is not related to dietary intake. However, potassium showed no change in serum concentration from the beginning to the end of the study conducted. Importantly, there are few studies related to the use of cottonseed in cattle diet and the serum biochemical indicators studied.

Considering the normal reference ranges suggested by Kaneko *et al.* (2008) (9.7 to 12.4mEqL^{-1}), the blood levels of Ca were considered subnormal in the beginning of the experiment (7.9 to 8.6mEqL^{-1}). However, at the end of the period studied (85 days), the animals fed diets containing 4.44 and 8.88% of cottonseed showed normal values while the others had results below the reference values (9.12 to 10.08 mEqL^{-1}) but they were very close to normal values (Tab. 2). According to Boin (1985), the Ca concentration in blood serum does not present great variation, because this decrease in blood Ca level increases the secretion of parathyroid hormone that, consequently, stimulates bone resorption and activation of vitamin D_3 (1.25-dihydroxycholecalciferol), decreasing urinary excretion of Ca and increasing intestinal absorption. It is debatable whether the total Ca content must be used in the diagnosis of disability, since the ionized Ca is actually the fraction which represents the amount available to the animal. However, research has shown that 50% of the total Ca is complexed (non-ionizable) with proteins (mainly albumin) and other organic substances, and the remainder is in ionized form (Morais *et al*., 2000). This may be an explanation for the cases of hypocalcaemia observed in this study.

In relation to serum phosphorus, and considering the reference ranges suggested by Kaneko *et al.* (2008), the animals were with subnormal serum levels at the beginning of the experiment (4.27 to 5.00mEqL^{-1}) (Tab. 2). At the end of the experimental period, the control treatment and treatments with 2.22; 6.66 and 11.11% of whole cottonseed in diets showed results slightly above the reference range (6.85, 6.75, 6.86 and 6.56mEqL^{-1} respectively). The diagnosis of mineral deficiencies is given by the serum levels of calcium and phosphorus. However, it is known that such levels can remain at a normal range for a long period after the cattle have been exposed to a serious deficiency of these elements (Balarin *et al.* 1992). In this study, the values found for Ca and P showed some results that were outside the normal range. However, the means of Ca and P values showed no difference ($P>0.05$) between treatments in the experimental period.

Considering the normal variation of K suggested by Kaneko *et al.* (2008) (3.9-5.8mEq/L), the animals were normokalemic at the beginning (4.57 to 5.20 mEqL^{-1}) and at the end of the experiment (4.83 to 5.15mEqL^{-1}) (Tab. 2). There was no effect ($P> 0.05$) of the addition of cottonseed in feed on this blood parameter. Regarding serum Fe, the values found in all treatments, at the beginning (164.00 to 227.00µg/dL) and at the end of the experimental period (258.75 to 331.33µg/dL) (Tab. 2), were above normal reference ranges (57-162µg/dL). As the control treatment also showed values above the reference range at the beginning and end of the experimental period, this change cannot be attributed to the diet with cottonseed. Studies have shown that blood elements of healthy animals may have values that differ according to age, race, and handling to which animals are subjected (Kappel *et al*, 1984; Kweon *et al*., 1986; Kohayagawa, 1993; Pogliani and Birgel Junior, 2007).

Considering the reference values suggested by Kaneko *et al.* (2008), the values found for Na at the beginning of experimental stage were normal. At the end of the experiment, the control treatment and those that used 2.22, 4.44 and 8.88% of cottonseed in diets were slightly higher, respectively, (154.25, 153.75, 155.60 and 155.00) from the reference values (132-152mEqL^{-1}) (Tab. 2). Sodium is essential for the membrane potential, which is fundamental for many specialized cell functions, such as muscle contraction, nerve impulse transmission, among others. The maintenance of body concentration is controlled only by the intake and excretion (Dibartola, 2000; Cunningham, 2004). Regarding magnesium concentrations, all cattle presented normal ranges at the beginning of the experiment (1.80 to 2.03mEqL^{-1}). At the end of the trial period, the control treatment and treatments with 2.22 and 8.88% of cottonseed in diets values were slightly above the reference values for Mg, 2.53, 2.55 and 2.45mEqL^{-1}, respectively (Tab. 2). However, both levels of Na and Mg showed no difference (P>0.05) between treatments.

With respect to serum chloride, considering the normal range suggested by Kaneko *et al*. (2008) (97-111mg/dL) at the beginning of the experiment, the control treatment and the treatments with 4.44 and 11.11% of cottonseed in diets showed results slightly below the normal range 95.33, 96.00 and 93.67, respectively, while the other treatments had normal values. At the end of the experimental period, all treatments showed results within the normal range (Tab. 2). However, these differences did not differ (P>0.05) between treatments.

Serum cholesterol levels, triglyceride and HDL at the beginning (B) and in the end (E) of the experimental period showed no difference (P>0.05) between treatments (Tab. 3). However, when comparing the concentrations of biochemical indicators from the beginning to the ones found at the end of the experiment, within each group, it was observed that, in relation to cholesterol, there was a significant increase (p<0.05) on the concentrations of groups receiving diets containing 8.88 and 11.11% of cottonseed. Regarding the triglyceride, the group that received a diet containing 4.44% of cottonseed also increased significantly (P<0.05) the serum levels. HDL concentrations of the groups fed diets containing 2.22 and 11.11% of cottonseed also showed a significant increase (P<0.05).

Table 3. Means and standard deviations (SD) of the serum cholesterol levels (COL), triglyceride (TG), high density lipoprotein (HDL), very low density lipoprotein (VLDL) and low density lipoprotein (LDL) of Nellore bulls fed diets containing different levels of cottonseed at the beginning (B) and end (E) of the experimental period (85 days)

Variable	Whole cottonseed in diet (kg/100kg)*					
(mg/dL)	0	2.22	4.44	6.66	8.88	11.11
COL(B)	98.33±26.31	112.00±9.54	118.67±34.06	119.33±10.69	103.50±19.74^{a}	126.67±9.50^{a}
COL(E)	142.50±22.63	185.00±35.68	187.00±32.71	188.20±44.70	175.25±31.53^{b}	192.40±12.91^{b}
TG(B)	15.33±4.04	16.33±3.21	17.33±7.51^{a}	18.00±6.93	24.75±5.90	18.67±2.89
TG(E)	24.50±5.80	22.25±6.55	28.80±17.40^{b}	21.80±6.30	23.25±4.86	21.80±4.09
HDL(B)	58.00±12.77	56.67±6.43^{a}	56.00±5.29	60.33±6.11	60.00±11.63	53.33±9.71^{a}
HDL(E)	79.00±7.39	88.50±19.05^{b}	84.40±19.02	92.60±41.75	98.50±22.75	93.40±20.86^{b}
VLDL (B)	3.20±0.62	3.27±0.64	3.47±1.50	3.60±1.13^{a}	4.95±1.18	3.60±0.54
VLDL (E)	4.90±1.00	4.67±1.51	4.73±1.86	4.80±0.90^{b}	4.65±0.97	4.20±0.85
LDL (B)	39.30±10.88	52.07±6.13	59.20±29.62	52.65±15.20	38.55±9.52	69.15±9.46
LDL (E)	58.60±23.58	97.00±27.98	108.87±31.31	102.95±30.44	72.10±28.18	90.55±26.68

a,b: different letters in the same column denote significant differences (P<0.05) between the results from the beginning compared to the end of the experiment, within each group - Paired *t* Student Test.
* In ANOVA treatments with different cottonseed concentrations showed no significant difference (P>0.05).

Cholesterol levels ranging from 98.33 to 126.67 mg/dL at the beginning of the treatment proved to be within the normal range described by Kaneko *et al*. (2008) (80 to 120mg/dL), with the exception of the treatment with 11.11% of cottonseed, which showed a value slightly higher than the limit of normality (126.67mg/dL). However, in the end of the experiment all treatments showed high serum levels (Tab. 3). As the control treatment also had an increase on cholesterol levels, this event in the end of the experiment cannot be attributed to diets containing cottonseed, may it be related to the increasing age of the cattle in the end of the experiment, as suggested by Kappel *et al.* (1984), that had previously pointed out that age is an important factor in the assessment of cholesterol levels in the blood.

Pogliani and Birgel Junior (2007) found that serum triglyceride levels are higher in younger animals, 16.30 to 34.80mg/dL, and 14.90 to 24.00mg/dL for animals over 48 months old, which is consistent with the results found in this study, where the values for serum TG ranged at the end of the experimental period between 21.80mg/dL to 28.80mg/dL. According to Kaneko *et al.* (2008), the values found for TG at the beginning and end of the experimental period were above the reference range (0 to 14mg/dL). However, the averages found for serum triglycerides presented no differences ($P>0.05$) between treatments, showing that they do not suffer influences of the diets during the experimental period. In ANOVA test, the values found for VLDL and LDL at the beginning and at the end of the experiment showed no significant difference ($P>0.05$). In Paired *t* Student Test, only the group that received a diet containing 6.66% of cottonseed showed significant difference ($P<0.05$) when comparing the averages at the beginning with those at the end of this study. However, plasmatic concentrations of VLDL and LDL were not influenced by the diet (Tab. 3).

Serum lipid values are increasingly used due to their value in prognosis of diseases. Blood biochemical composition accurately reflects the metabolic status of the animal tissues, and may evaluate tissue lesions, disorders in the functioning of organs, animal adaptation to the physiological nutritional challenges and specific metabolic imbalances (González and Scheffer, 2002). Coppo *et al.* (2003) evaluated calves that were 2 and 6 months old and finding values for HDL 109mg/dL and 113mg/dL, respectively. The values found in this study to HDL ranged from 53.33 to 60.33 at the beginning and 79.00 to 98.50mg/dL at the end of experimental period.

CONCLUSIONS

The use of cotton seed up to 1.13kg/animal/day, which resulted in consumption of 5.05g of free gossypol/animal/day, did not cause significant changes in the concentrations of blood parameters analyzed in this study, with the exception of magnesium that may have increased its bioavailability with the use of diets containing up to 6.66% of cottonseed.

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